RESEARCH ARTICLE

Cascade Dissociations of Peptide Cation-Radicals. Part 1. Scope and Effects of Amino Acid Residues in Penta-, Nona-, and Decapeptides

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Abstract

Amino acid residue-specific backbone and side-chain dissociations of peptide z ions in MS^3 spectra were elucidated for over 40 pentapeptides with arginine C-terminated sequences of the AAXAR and AAHXR type, nonapeptides of the AAHAAXX"AR and AAHAXAX"AR type, and AAHAAXX"AAR decapeptides. Peptide z_n ions containing amino acid residues with readily transferrable benzylic or tertiary β -hydrogen atoms (Phe, Tyr, His, Trp, Val) underwent facile backbone cleavages to form dominant z_{n-2} or z_{n-3} ions. These backbone cleavages are thought to be triggered by a side-chain β -hydrogen atom transfer to the z ion C_α radical site followed by homolytic dissociation of the adjacent C_α –CO bond, forming x_{n-2} cation-radicals that spontaneously dissociate by loss of HNCO. Amino acid residues that do not have readily transferrable β -hydrogen atoms (Gly, Ala) do not undergo the $z_n \rightarrow z_{n-2}$ dissociations. The backbone cleavages compete with side-chain dissociations in z ions containing Asp and Asn residues. Side-chain dissociations are thought to be triggered by α -hydrogen atom transfers that activate the C_β – C_γ or C_β –heteroatom bonds for dissociations that dominate the MS^3 spectra of z ions from peptides containing Leu, Cys, Lys, Met, Ser, Arg, Glu, and Gln residues. The Lys, Arg, Gln, and Glu residues also participate in γ -hydrogen atom transfers that trigger other side-chain dissociations.

Key words: Electron transfer dissociation, Peptide z ions, Cascade dissociations

Introduction

The electron-based dissociation techniques of mass spectrometric peptide and protein sequencing rely on

ion–electron or ion–ion recombination resulting in charge reduction of the peptide ions by electron attachment. This electron attachment has two major effects. One effect is energetic, as the electron–ion or ion–ion recombination energy excites the charge-reduced peptide ion and drives its dissociations. The other effect is electronic, whereby the incoming electron perturbs the electronic structure of the charge-reduced ion and dramatically lowers bond dissociation and activation energies [1, 2]. The experimental methods utilizing electron attachment, electron capture

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dissociation (ECD) [3], and electron transfer dissociation (ETD) [4] differ in the extent of excitation delivered to charge-reduced peptide ions, which is reflected in the extent and even nature of fragmentations. The backbone fragmentations mainly proceed by competitive dissociations of bonds between the amide nitrogens and the C_α carbons of the adjacent amino acid residue, N– C_α cleavage for short, forming even-electron N-terminal fragments of the c -type and odd-electron C-terminal fragments of the z -type (Scheme 1). The combination of ion excitation by ECD and low dissociation energy raises the possibility that the primary c - and z -type fragment ions may undergo consecutive dissociations forming secondary products. Alternatively, stable primary c and z fragment ions produced by ETD can be selected by mass and collisionally activated to provide MS³ mass spectra. The main question concerning spontaneous or collisionally activated dissociations of c - and z -type fragment ions is the information content that can be mined from the MS³ spectra. Presumably, the c - and z -type fragment ions carry structure information about their inherent amino acid sequence. This would be valuable to extract, especially so in ETD of larger peptide ions where the backbone dissociations sometimes do not go beyond a few residues from the N- or C-terminus [5].

Consecutive dissociations on ECD were first identified by O'Connor and coworkers in a study of doubly protonated ions from cyclic peptides cyclo-LLFWAVGH, gramicidin S, and cyclosporin A [6]. ECD of these ions produced a number of fragments, some of which were formed by cross-ring cleavages, indicating dissociations of at least two bonds

in the peptide chain. Cross-ring cleavages have also been observed in ECD of copper complexes of diastereoisomeric cyclo-H β AHK peptides [7]. These cross-ring fragmentations contrast the well known resistance of ring cleavage in proline residues [8] that results in a “proline stop” in peptide sequencing by ECD or ETD. O'Connor et al. suggested reaction mechanisms for the observed dissociations that involved initial N– C_α bond cleavage in the ring, forming C_α radicals analogous to z fragment ion [6]. These were presumed to attack one of the amide nitrogens to initiate another N– C_α ring cleavage, leading to fragmentation.

Collisional activation of ETD product ions has been reported by the McLuckey [9, 10] and Coon groups [11]. In particular, z -type ions were found to undergo radical-induced dissociations in the MS³ mass spectra that produced sequence fragments of the z type. In contrast, several z -type ions from ETD of pentapeptide ions of a nontryptic type showed no $z_n \rightarrow z_m$ transitions, while other radical-induced dissociations were prevalent [12]. To gain insight into the nature of these dissociations and to assess their general utility for peptide sequence analysis, a more detailed study dealing with the dissociation energetics, kinetics, and specificity for particular amino acid residues appeared to be warranted. We now present a systematic study of several pentapeptides of the AAXAR type in which we placed different amino residues (X = A, C, D, F, G, H, K, L, M, N, S, R, V, W, and Y) in the sequence. With regard to the specific effects of histidine residues on peptide ECD and ETD [13], we also investigated a series of AAHXAR peptide ions where X were the amino acid residues listed above. To assess the size effect on z ion dissociations [14], we investigated a series of AAHAAXX"AR, AAHAXAX"AR, and AAHAAXX"AAR peptides where X and X" were combinations of D, E, N, or Q. All these sequences anchor one positive charge at the protonated C-terminal arginine residue to provide abundant z -type fragment ions upon ETD. In this paper, Part 1, we investigate the effects of amino acid residues on promoting backbone and side-chain dissociations upon collisional activation of mass-selected z ions. In the accompanying paper, Part 2, we report studies of z ion dissociations using infrared multiphoton dissociation (IRMPD) and analysis of the reaction energetics and kinetics using electronic structure theory calculations with the aim of assigning plausible reaction mechanisms for z ion cascade dissociations. The results reported in these two studies provide a coherent explanation of the effects of amino acid residues on the peptide cation-radical dissociations.

Scheme 1. Formation of c and z fragment ions by ETD

Experimental

Materials and Methods

All peptides were custom-synthesized by NEOPeptide Laboratories (Cambridge, MA, USA) or CHI Scientific (Maynard, MA, USA) and used as received. ETD mass spectra were measured on a Thermo Fisher (San Jose, CA,

USA) LTQ XL linear ion trap instrument, outfitted with a chemical ionization source for the production of fluoranthene anion radicals as ETD reagent. Selected measurements were carried out on a Thermo Fisher LTQ Orbitrap Velos hybrid mass spectrometer equipped with an ion source for ETD. Peptide solutions (10 μ M) in 50/50/1 water/methanol/acetic acid were electrosprayed to form precursor cations for ETD. Electrospray ionization of the pentapeptides produced mainly doubly protonated ($M + 2H$)²⁺ ions and minor singly protonated ($M + H$)⁺ ions, typically in a $\geq 10:1$ ratio. Electrospray of the nona- and decapeptides produced triply protonated ($M + 3H$)³⁺ ions and doubly protonated ($M + 2H$)²⁺ ions, typically in a $\geq 4:1$ ratio. Precursor ions were isolated with a window of 3–4 m/z units to accommodate nearest ¹³C isotopologues and allowed to react with fluoranthene anions for 100, 200, or 300 ms under the above-described conditions.

Results

Survey of ETD and MS³ Spectra

The basic ETD experiments that we performed with the entire series of penta-, nona-, and decapeptides are illustrated with ions from the pentapeptide AAWAR (Figure 1a–c). ETD of the (AAWAR + 2H)²⁺ precursor ions (m/z 287.6584) generated backbone **z**₁ through **z**₄ fragment ions, in addition to forming weak charge-reduced ions (m/z 575.3166), which were distinguished from the ¹³C isotope of the ($M + H$)⁺ ion at m/z 575.3113. The other fragment ions were at m/z 574 by loss of a hydrogen atom, m/z 558 by loss of ammonia, and m/z 532, 516 by losses of side-chain groups from the Arg residue (Figure 1a). The **z** ions were mass-selected and subjected to collisional activation, which generated MS³ spectra, as shown for the **z**₅ and **z**₄ ions in

Figure 1. (a) ETD mass spectrum of the (AAWAR+2H)²⁺ ion at m/z 287.66. (b) CID-MS³ mass spectrum of the ETD **z**₅ fragment ion at m/z 558. (c) CID-MS³ mass spectrum of the ETD **z**₄ fragment ion at m/z 487

Figure 1b, c, respectively. It should be noted that the z_n ions, where n is the number of amino acid residues in the precursor peptide ion, are fragments formed by elimination of ammonia upon ETD. Although the mechanism for the formation of these ions is different from that leading to backbone z fragment ions [15] previous ^{15}N labeling studies established that the z_n ions originated by specific loss of the N-terminal amino group [16] and likely were N-terminal C_α radicals homologous to the shorter backbone z fragment ions. We use the z_n ions ($n=5, 9$, and 10 for the penta-, nona-, and decapeptides, respectively) as reference species in evaluating the MS^3 fragmentations of the sequence z ions.

The MS^3 spectrum of the z_4 ion (m/z 487.2529) in Figure 1c shows a predominant dissociation leading to the z_2 fragment ion that amounts to 51 % of the sum of all fragment ion intensities. In contrast, the z_3 (1.4 %) and z_1 (0.2 %) fragment ions are very weak. The identity of the z_2 ion was supported by accurate mass measurements that gave m/z 230.1372 to be compared with m/z 230.1373 calculated for $\text{C}_9\text{H}_{18}\text{N}_4\text{O}_3^+$. The main competing dissociations of the z_4 ion were losses of a $\text{C}_3\text{H}_6\text{NO}$ fragment from the deaminated N-terminal Ala residue, forming the fragment ion at m/z 415.2081, and the Trp $\text{C}_9\text{H}_7\text{N}$ side chain, giving rise to the fragment ion at m/z 358.1954 (Figure 1c). The facile $z_4 \rightarrow z_2$ dissociation was not confined to Trp containing precursor ions, as several other pentapeptide sequences showed a similar effect. This prompted us to carry out an extensive investigation of the effects of amino acid residues on the cascade dissociations of z ions.

In contrast, the MS^3 spectrum of the z_5 ion (m/z 558.2897, Figure 1b) showed much more diverse dissociations, including a major loss of the Trp side chain (m/z 429.2329, loss of a $\text{C}_9\text{H}_7\text{N}$ neutral molecule) and stepwise fragmentations of the Ala residues (m/z 488.2610 by loss of a $\text{C}_3\text{H}_4\text{NO}$ radical, and 417.2240 by loss of a $\text{C}_6\text{H}_9\text{N}_2\text{O}_2$ radical). The z_2 ion at m/z 230.1371 amounted to 8.4 % of the ETD fragment ion intensities (Figure 1b).

AAXAR Peptides

Figure 2a shows a plot of z_2 fragment ion relative intensities in the MS^3 mass spectra of z_4 ions from ETD of 15 pentapeptides of the AAXAR type. We did not include in this set the Pro (ring structure), Thr (similar to Ser), Ile (similar to Val), Glu, and Gln residues. The distribution shows two groups of amino acid residues (X). One group, comprising of Val, Asn, Asp, His, Phe, Tyr, and Trp, gave high (22 %–67 %) z_2 ion relative intensities, expressed as percent of the sum of fragment ion intensities. For Val, His, Phe, Tyr, and Trp, this indicates a very facile, dominant dissociation leading to the z_2 fragments. The second group, comprising of Gly, Ala, Ser, Cys, Leu, Met, Lys, and Arg, gave z_2 fragments of low to very low relative intensity, indicating that this backbone dissociation competed poorly with other z_4 ion dissociations. The nature of the competing dissociations depended on the amino acid residue. The MS^3

spectra of z_4 ions from peptides containing Cys, Leu, Met, Lys, and Arg were dominated by side-chain fragmentations, which are typical for these residues [17]. Figures S1–S5 (Supplementary Data) show dominant losses of $\text{HS}^\bullet$ (m/z 371) and CH_2S (m/z 358) from Cys (Figure S1), $\text{CH}_3\text{SCH}_2^\bullet$ from Met (Figure S2), $\text{C}_3\text{H}_7^\bullet$ and C_4H_8 from Leu (Figure S3), $\text{C}_2\text{H}_5\text{N}$, $\text{C}_3\text{H}_8\text{N}^\bullet$, and $\text{C}_4\text{H}_9\text{N}$ from Lys (Figure S4), and $\text{C}_3\text{H}_8\text{N}_3^\bullet$ and $\text{C}_4\text{H}_9\text{N}_3$ from Arg (Figure S5). These are all radical-induced dissociations triggered by transfers of hydrogen atoms from C_α or C_β positions [12, 18]. Side-chain dissociations were also observed for z_4 ions from Asn (loss of $^\bullet\text{CONH}_2$, 64 %) and Asp (loss of CO_2 , 33 %), although peptides containing these residues also showed abundant competing back-bone dissociations leading to z_2 ions.

The z_4 ions from peptides containing the Gly and Ala residues did not give any fragment ions that could be assigned to side-chain dissociations. An interesting feature of these mass spectra are dissociations leading to a series of ($y_n - 2\text{H}$) ions (e.g., at m/z 173, 244, and 315 for AAAAR).

The backbone dissociations of z_4 ions having the Val, Asn, Asp, His, Phe, Tyr, and Trp are highly specific for the formation of z_2 ions. Figure 2b shows very inefficient formation of z_3 ions that are <2 % in the MS^3 mass spectra. The only apparent exception is the z_4 ion from AAKAR, which gives a fragment ion (m/z 358) that is at the same nominal m/z as a z_3 ion. However, the formation of this ion can be explained by an elimination of a $\text{C}_4\text{H}_9\text{N}$ neutral fragment from the Lys side chain, which is consistent with the eliminations of homologous $\text{C}_2\text{H}_5\text{N}$ and $\text{C}_3\text{H}_8\text{N}^\bullet$ fragments (Figure S4).

The above-described pattern of z_4 ion dissociations is largely maintained in dissociations of the corresponding z_5 ions (Figure 2c). These are the products of ammonia loss upon ETD of the precursor ions and their presence in the spectra provides no sequence information. The MS^3 spectra of z_5 ions show moderately abundant formation of z_2 ions for the peptide sequences involving the Val, Asn, Asp (in the form of a $z_2 + \text{H}$ ion), His, Phe, Tyr, and Trp residues. Overall, the $z_5 \rightarrow z_2$ dissociations are about 3–4 times less abundant than the pertinent $z_4 \rightarrow z_2$ ones. A larger drop is seen for His. The $z_5 \rightarrow z_2$ dissociations retain some specificity compared with the $z_5 \rightarrow z_3$ dissociations, which give rise to <2 % z_3 ion intensities. A sole exception is the Arg-containing z_5 ion, which gives a moderately abundant $z_3 + \text{H}$ fragment (Figure 2d).

AAHXR Peptides

The Figure 2 data indicated specific dissociations occurring in the z_4 and z_5 ions at the C-terminal side next to the Val, Asn, Asp, His, Phe, Tyr, and Trp residues. It was therefore of interest to investigate the effect of the amino acid residue in the adjacent position, which was Ala in the above-described AAXAR set. This was studied for a set of peptides with AAHXR sequences, where X was A, C, D, F, G, H, K, L, M, N, S, R, V, W, and Y. Figure S6A shows the relative

Figure 2. Relative intensities (% of sum of fragment ion intensities) in the ETD-CID-MS³ spectra of fragment ions from AAXAR peptides: (a) z_2 from z_4 , (b) z_3 from z_4 , (c) z_2 from z_5 , (d) z_3 from z_5

abundances of z_2 fragment ions in the MS³ spectra of z_4 precursors. For most amino acid residues, the $z_4 \rightarrow z_2$ dissociations remain the predominant fragmentations, similar to that from AAHR. However, comparison of the Figure 2a and Figure S6A data indicates that the z_2 ion relative intensity in the MS³ spectra of z_4 ions from AAHR are, on the whole, somewhat lower than for AAHR, owing to several factors.

A major factor is the presence of Cys and Met residues in AAHR that each greatly diminished the probability for $z_4 \rightarrow z_2$ dissociation. The reasons are competing side-chain dissociations in Cys and Met that dominate the fragmentation. This effect is less severe for other residues with dissociation-prone side-chains (e.g., Leu, Ser, Lys, and Arg, which compete with the His-promoted backbone cleavage.

Another, albeit less severe, effect is due to consecutive dissociations of z_2 ions. This is mainly seen for Asn and Asp, which undergo consecutive losses of CONH₂ and CO₂, respectively, from the side chains to give the m/z 230 ions.

The third effect is illustrated with the z_4 ion from AAHR, which contained two strongly enhancing residues and yet gave only a moderate z_2 ion relative intensity (Figure S6A). The MS³ spectrum showed two backbone fragments (Figure 3). The more abundant one at m/z 388.1844 was formed by C_α-CO bond cleavage at the His residue, as supported by accurate mass measurements. Elimination of HNC(43.0058 Da) from the m/z 388 ion then formed the z_2 ion at m/z 345.1786. This sequence was corroborated by an MS⁴ spectrum of the m/z 388 ion, which showed a dominant loss of a 43 Da neutral fragment. This indicated that the selective formation of the z_2 ions may proceed in two steps also in the other peptides. Indeed, inspection of the MS³ spectra showed low-to-moderate intensity $z_2 + 43$ ions from AAHR, AAWAR, AAYAR, AAVAR, AAHR, and other sequences giving prominent z_2 ions. The peculiar feature of these intermediate fragment ions was that they appeared at m/z values that were 0.2–0.3 units lower than the expected one (e.g., m/z 273.1 from AAXAR) and showed

Figure 3. CID-MS³ mass spectrum of the ETD z_4 fragment ion at m/z 553 from AAHW

irregular shapes tailing towards lower m/z (Figure 1c). Such behavior would be expected if the m/z 273 intermediates were metastable and dissociated during ejection from the ion trap [19, 20]. The kinetics of backbone dissociations forming z_2 ions and competing side-chain dissociations will be addressed in Part 2 of this work using energy- and time-resolved infrared multiphoton dissociation.

Peptides with C-Terminal Lysine

The MS³ dissociations of arginine C-terminated z_4 and z_5 ions showed mainly radical-induced reactions, whereas proton-induced dissociations leading to b - and y -type ions were minor. Presumably, this may be due to the high basicity of the arginine side chain that sequesters the proton and thus increases the activation energies for proton transfer to amide groups, which is needed for charge-driven backbone dissociations [21]. Therefore, it was of interest to investigate z ions C-terminated with the less basic lysine residue to establish if charge-driven backbone dissociations would become competitive with the radical-induced dissociations.

Figure 4 shows the MS³ spectra of lysine-C-terminated z_4 ions from doubly charged AAHK, AAHAK, AHAHK, and HAAAK. The spectra of $^{\bullet}$ AAHK, $^{\bullet}$ AHAK, and $^{\bullet}$ HAHK z_4 -ions showed fragment ions at m/z 352 that were formed by loss of a C_3H_8N radical from the lysine side chain. These dissociations required cleavage of the C_{α} - C_{β} bond in the lysine side chain and proton migration from the lysine ϵ -ammonium group to another basic group, presumably the histidine imidazole ring. Note that loss of C_3H_8N is much less abundant from $^{\bullet}$ AAAK which lacks the basic group. The dissociations of $^{\bullet}$ AHAK and $^{\bullet}$ AAAK were analogous to those of the arginine C-terminated ions. The MS³ spectrum of $^{\bullet}$ AHAK showed a major z_2 ion at m/z 202. The MS³ spectrum of $^{\bullet}$ AAAK showed a major peak at m/z 287, which corresponds to a fragment ion by loss of a C_3H_5O radical and is analogous to the ($y_3 - 2H$) ion at m/z 315 from $^{\bullet}$ AAAR. In summary, substituting a C-terminal Lys for Arg did not have a substantial effect on the fragmentations of the z_4

ions containing histidine. A major $z_4 \rightarrow z_2$ dissociation observed for $^{\bullet}$ AHAK was analogous to that described for $^{\bullet}$ AHAR. The radical-induced dissociations of the Lys terminated z_4 ions outcompeted the charge-driven dissociations.

Nona- and Decapeptides

The MS³ spectra of the z_4 and z_5 ions from several pentapeptides identified the V, N, D, H, F, Y, and W amino acid residues as enhancing specific peptide backbone cleavages. It was therefore of interest to investigate the dissociations of larger peptide ions containing one or more enhancing groups to elucidate if the effect was present and to what extent. We investigated three series of His-containing peptides with the AAHAAXX"AR, AAHAXAX"AR, and AAHAAXX"AAR sequences, where X and X" were combinations of D, E, N, and Q residues.

ETD spectra of the doubly protonated ions from these peptides showed very substantial charge-reduced $(M + 2H)^{+\bullet}$ ions. This was also observed for the ETD spectra of triply protonated ions which showed the $(M + 3H)^{+}$ and $(z_{n-2} + H)$, $(z_{n-1} + H)$, and $(z_n + H)$ fragment ions containing the reduced histidine radical residue, while the lower z ions not containing the His residue did not pick up hydrogen atoms. These effects are illustrated with the ETD spectra of triply and doubly protonated AAHAANNAAR (Figure 5a and b). ETD of the doubly protonated ion mainly formed z series fragment ions (Figure 5b). The presence of an extra proton in the triply protonated precursor ion gave rise to a series of abundant c_3 through c_8 ions upon ETD (Figure 5a). Note that the $(z_8 + H)$ ion at m/z 809.4012 has the same elementary composition as the c_9 fragment ion and so these two fragment ions cannot be distinguished in the ETD mass spectra. The ETD spectra of the triply protonated ions showed the complementary c_n and z_{10-n} ions of closely matching intensities for $n=3-8$ (e.g., c_3 and z_7 , c_4 , and z_6 , etc. (Figure 5a). This interesting phenomenon is presumably due to the mode of c and z ion formation. The z ions contain the charged arginine residue but their formation does not require any hydrogen atom transfers. The c ions are formed by a proton transfer to the amide group [22]. The match of the c_n and z_{10-n} ion intensities implies that (1) the proton being transferred to the c_n ions must originate from the N-terminus or histidine charged groups, and (2) the charge reduction leading to backbone cleavages does not occur in the arginine residue. Electron attachment to the arginine residue manifests itself by other dissociations (e.g., loss of guanidine (m/z 909 and 908 in Figure 5a and b, respectively). This is consistent with the chemistry of guanidinium [23], arginine radicals [24], and cation-radicals [25].

An interesting feature of the ETD spectra of the doubly protonated ions was the presence of $z_n + H$ ions that were formed by backbone cleavages in the vicinity of the D, N, Q, and E residues (Figure 5b). The formation of $z_n + H$ ions was both position and residue dependent. The most abundant formation of $z_n + H$ ions was observed for the dissociations in AAHAAXX"AAR that occurred at or between the polar

Figure 4. CID-MS³ mass spectra of the ETD z_4 fragment ions from AAAHK, AAHAK, AHAAK, and AAK

residues, $XX'' = DD, NN, DN$, and ND . Figure S7 (Supplementary Data) shows that $z_4 + H$ ions represented 48 %–65 % of the z_4 group fragments, while $z_5 + H$ ions made 50 %–58 % of the z_5 group fragments. The E and Q residues were less effective in mediating the $z_4 + H$ (15 %–28 %) and $z_5 + H$ (16 %–26 %) fragment formation. The relative intensity of $z_n + H$ ions rapidly decreased in fragmentations occurring farther away from the D, N, E, and Q residues. For example, the z_6 group of fragment ions by cleavage between the Ala-Ala residues showed <10 % of $z_6 + H$ ions, and the $z_7 + H$ ions next to the abundant z_7 ions were nearly absent (< 2 %). All these figures refer to $z_n + H$ ion intensities that were corrected for the natural abundance of the ^{13}C , ^{15}N isotope satellites of the pertinent z_n ions.

The formation of $z_n + H$ ions by hydrogen atom transfer between the incipient c_m and z_n fragments is facilitated by

noncovalent interactions holding these fragments before they separate [26, 27]. Presumably, the polar D, N, E, and Q residues provide hydrogen bonding interactions allowing the c_m and z_n fragments to undergo exothermic hydrogen atom transfer in intermediate complexes formed by N- C_α bond cleavage. This is tentatively sketched in Scheme 2, although exploring the mechanistic details and explaining the differences between the D and N residues on one hand and E and Q on the other would require a dedicated study.

The MS³ dissociations of z_n ions were studied for $n=3$ –9 from AAHAAXX"AR and AAHAXAX"AR and for $n=6$ –10 for AAHAAXX"AAR peptides. The results for the decapeptides are illustrated in Figure 6 with dissociations of z ions from AAHAADDAAR and AAHAEEEAAR. The z_{10} ions from both peptides showed rather nonspecific backbone cleavages whereby the z ions were accompanied

Figure 5. ETD mass spectra of (a) triply protonated and (b) doubly protonated AAHAANNAAR ions

by $z + 1$ and $z - 1$ fragments. The z_9 ions showed highly specific cleavage next to the His residue giving abundant z_7 fragment ions that accounted for >20 % of the fragment ion intensities. This dissociation is analogous to the formation of the abundant z_2 ions from AAXAR (X = W, F, Y, H, V) and AAHXR peptides, as described above, and points to the backbone cleavage enhancing effect of the His residue. MS³ of the z_7 and z_6 ions not containing His showed differences between the DD and EE containing peptides. The DD containing z_7 and z_6 ions underwent backbone cleavage next to either Asp residue, giving rise to prominent z_4 and z_3 fragment ions (Figure 6). The main competing dissociation was loss of COOH (45 Da) from the Asp side chain. The MS³ spectra of the DD containing z_7 and z_6 ions pointed to the mild effect of the Asp residues in enhancing backbone dissociations that was analogous to that described above for AADAR. The EE containing z_7 and z_6 ions gave only very

weak backbone fragments, the main dissociations being side-chain cleavages leading to loss of CH₂COOH (59 Da) and CH₂=CHCOOH (72 Da) neutral fragments. It is noteworthy that these radical-induced dissociations overwhelmingly outcompeted charge-induced dissociations (e.g. the otherwise facile backbone cleavages next to Asp that formed only very weak y_3 (m/z 317) and y_4 (m/z 432) ions.

The MS³ spectra of z ions from the other decapeptides were quite analogous to those from AAHAADDAAR and AAHAEEAAR. All z_9 ions showed the highly specific cleavage next to the His residue giving abundant z_7 fragment ions. The z_7 and z_6 ions from the ND, DN, and NN containing peptides further gave z_4 and z_3 fragment ions owing to the backbone cleavage-enhancing effect of these residues. The z_7 and z_6 ions from the QE, EQ, and QQ containing peptides mainly showed radical-induced side-chain losses of CH₂COOH, CH₂CONH₂, CH₂=CHCOOH, and CH₂=CHCONH₂.

Scheme 2. Possible mechanisms for the formation of $z_5 + H$ and $z_4 + H$ ions by hydrogen atom migrations in ion-molecule complexes

The MS^3 spectra of the z ions from the several nonapeptides showed the same regular behavior. Figure S8 shows the enhancement of the backbone cleavage next to the His residue in the z_8 ion from AAHAANNAR, giving an abundant z_6 secondary fragment ion. Analogous dissociations next to the His residue were observed for z_8 ions from all nonapeptides in our set. The z_6 and z_5 ions from AAHAAX-X"AR where X, X" = D, N also dissociated by backbone cleavage between the Asp and Asn residues giving abundant z_3 fragment ions, which documented the enhancing effect of Asp and Asn (Figure S8). By contrast, z_6 and z_5 ions from EE, QE, EQ, and QQ containing nonapeptides underwent only side-chain dissociations. The relative enhancing effect is illustrated with dissociations of z ions from AAHANADAR, AAHADADAR, and AAHADAEAR. The z_6 and z_4 ions from the NAD, DAD, and NAN-containing peptides show abundant $z_6 \rightarrow z_4$ and $z_4 \rightarrow z_2$ dissociations occurring next to the D or N residues. The $z_6 \rightarrow z_4$ dissociations are also abundant in the MS^3 spectra of the DAE-containing ions, but not the EAE, EAQ, QAE, and QAQ-containing ones (Figure S9, Supplementary Data). Figure S10 shows the MS^3 spectra of the z_6 ion (m/z 616) from AAHAEDAR (top) and AAHADAEAR (bottom). The backbone-cleavage enhancing effect of the Asp residue is quite visible, promoting the formation of the z_2 ion (m/z 230) in the top spectrum and the z_4 ion (m/z 430) in the bottom spectrum, both corresponding to dissociations next to the Asp residue. In contrast, dissociations next to the Glu residue are substantially less abundant. Both MS^3 spectra show competitive radical-induced losses of side-chain groups from the Glu (m/z 557, loss

of CH_2COOH , and m/z 544, loss of $CH_2=CHCOOH$) and Asp (m/z 572, loss of CO_2) residues.

Discussion

Backbone Cleavages

The MS^3 spectra of z ions from our series of penta-, nona-, and decapeptides indicated that the H, V, N, D, F, Y, and W amino acid residues enhanced backbone-cleavages at their C-terminal side. A common structural feature of these residues is that they all have readily abstractable hydrogen atoms in the β -position in the side chain. These are secondary hydrogen atoms of a benzylic or heterobenzylic type in H, F, Y, and W, a tertiary hydrogen atom in V, and secondary hydrogen atoms next to carbonyl groups in D and N. Such hydrogen atoms are prone to radical abstractions [18] that are thermochemically facilitated by the stability of the radical intermediates formed (e.g., A_1 and A_2 from His (Scheme 3)). An equally important feature is that the side-chain structures in radical intermediates of this type do not allow a facile β -fission of the side-chain group to proceed. Other dissociations involving the side-chain radicals, e.g., loss of H_α from all amino acid residues, or loss of a H_γ from Val do not seem to compete efficiently. The main dissociation of radical intermediates of type A is a C_α -CO bond cleavage leading to loss of N-terminal neutral fragments and forming the x ($z + HNCO$) radical intermediates (Scheme 3) [12]. These rapidly dissociate by elimination of HNCO to

Figure 6. Relative intensities (% of sum of MS^3 fragment ion intensities) of z fragment ions in the CID- MS^3 mass spectra of z_6 - z_{10} precursors from ETD of doubly protonated AAHAADDAAR and AAHAAEEAAR. The relative intensities are plotted on the same scale to allow rapid visual comparison. The dark brown bars indicate $z_n - 1$ ions. The green bars indicate $z_n + 1$ ions

form the z_{n-2} or z_{n-3} ions. The somewhat anomalous stability of the x_2 ion from AAHWR can be explained by side reactions of the radical intermediate with the Trp ring which is a known radical trap [28–30]. The reaction mechanism in Scheme 3 is similar to those suggested [10, 19] and computationally studied [12] previously. It comprehensively explains the chemistry of several amino acid residues in a

systematic way. Scheme 3 also provides a qualitative explanation of the more facile $z_n \rightarrow z_{n-2}$ dissociations compared with the $z_n \rightarrow z_{n-3}$ ones. The C_α radical in the position N-terminally adjacent to the H, V, N, D, F, Y, or W residues can transfer the β -H atom through a 6-membered transition state [12]. A prerequisite for this reaction is a $\text{trans} \rightarrow \text{cis}$ rotation of the pertinent terminal amide bond to allow

Scheme 3. H_β migrations in z ions

close approach of the C_α radical to the β -H atom in the transition state. Previous analysis of radical dissociations in z ions pointed out that amide rotation barriers in C_α -radical-carrying residues ($61\text{--}68\text{ kJ mol}^{-1}$) were notably lower than in regular peptide amide groups and comparable to the transition state energies for hydrogen atom migrations [12]. Thus, one can presume that the terminal amide rotation step in the z ion does not significantly affect the reaction kinetics. In contrast, β -H atom transfer to the more distal C_α -radical, as in the formation of the A_2 intermediate (Scheme 3), necessitates a 9-membered cyclic transition state, which can accommodate two trans-amide bonds only at the cost of substantial steric strain. Assuming that the amide rotation at the C_α -radical terminus is facile, the β -H atom transfer still involves a trans-amide bond of the inner amino acid residue, which imposes strain in the transition state and presumably increases its potential energy [12].

Interestingly, the radical-induced backbone dissociations in the A_1 and A_2 type intermediates do not involve the adjacent $\text{N}-C_\alpha$ bonds. This is clearly shown by the absence of z fragments terminated by H, V, N, D, F, Y, and W (e.g., for $z_5 \rightarrow z_3$ and $z_4 \rightarrow z_3$ transitions in the pentapeptides, $z_9 \rightarrow z_7$ and $z_8 \rightarrow z_7$ transitions in the nonapeptides, and $z_{10} \rightarrow z_8$ and $z_9 \rightarrow z_8$ transitions in the decapeptides). When such dissociations occur, albeit to a low extent, they are accompanied by hydrogen transfers forming $z + \text{H}$ and $z - \text{H}$ fragment ions (Figure 6). The rationale for the absence of $\text{N}-C_\alpha$ bond dissociations in z ions follows from the high potential energy of the neutral amide N-centered radicals, $\text{R}-$

$\text{CONH}^\bullet$, which would be formed by such a cleavage. A high-level computational study of small amide systems reported that amide N-centered radicals were $55\text{--}70\text{ kJ mol}^{-1}$ less stable than C_α radicals [31].

Peptide z ions containing Cys and Met residues showed very low to no propensity for backbone cleavages and instead dissociated by side-chain losses. This can be visualized (Scheme 4) as starting with a transfer of the H_α from the Cys residue, forming intermediate B_1 , which undergoes a β -fission elimination of a stable SH radical. The elimination of CH_2S can be initiated by the competing transfer of the sulfhydryl H atom (H_S) forming thiyl radical intermediate B_2 . Scheme 4 shows the transfer of the Cys H_α and thiol H_S to the terminal C_α radical side in the z ion. However, either of the H atoms presumably can also be transferred to the His C_β in intermediate A_1 (Scheme 3) to compete with the C_α -CO bond cleavage. It should be noted that the mechanisms of Cys H_α transfer call for cyclic transition states that cannot sterically accommodate a trans-amide bond at the His or other spacer residues and must involve trans $\rightarrow$ cis amide isomerization. The MS^3 spectra of the z_5 and z_4 ions from AAHCR show that the loss of SH vastly outcompeted the backbone cleavage. This indicates that the overall rate for the amide rotation, Cys H_α transfer, and SH loss is greater than that for the C_α -CO bond cleavage. The current experimental data do not allow us to identify the rate-determining steps in these dissociations. We will address this question and the detailed mechanism of this dissociation in Part 2 of this study.

Scheme 4. H_α and sulfhydryl hydrogen atom migrations in z ions

Side Chain Loss

An interesting feature of z ion dissociations is the competition between side-chain losses from the Glu and Gln residues, which are due to radical induced reactions. Loss of CH_2COX radicals ($X = OH, NH_2$) is presumably triggered by H_α abstraction from Glu and Gln, respectively, followed by β -fission in the side chain in a manner analogous to that shown for loss of $HS^\bullet$ from Cys (Scheme 4). Loss of $CH_2 = CHCOX$ can be visualized by an initial transfer of an H_γ atom from the side chain, triggering a β -fission in the intermediate (I , Scheme 5). Both types of dissociations

involve energetically plausible intermediates in which the radical site, C_α or C_γ , is stabilized by conjugation with the adjacent carbonyl group. By comparison, transfer of H_β from the inactivated methylene groups in the E and Q side chains is much less frequent, resulting in very minor eliminations of COX that occur in C_β radical intermediates formed by such H atom transfers. For example (Figure S9, Supplementary Data), the combined loss of COX (m/z 584, 585) from z_6 *AEAR and *AQEAR ions (both at m/z 629) was only 5 %–6 % of the combined loss of CH_2COX (m/z 570, 571) and $CH_2 = CHCOX$ (m/z 557, 558). Similar low ratios for COX loss were observed for the other combinations of E and

Scheme 5. H_γ migrations and side-chain loss from Glu and Gln residues in z ions

Q residues in z ions from nona- and decapeptides. Hence, the relative stability of the side-chain radical intermediates plays a role in the kinetics of side-chain dissociations in E and Q.

The energetically favored losses of CH_2COX and $\text{CH}_2 = \text{CHCOX}$ can be affected by the accessibility of the Glu and Gln H_α and H_γ atoms to the radical site, as distinguished by the competitive formation of the respective fragment ions, which are distinguished by mass in the MS^3 spectra. This is shown for dissociations of several z ions from AAHAAEQA(A)R and AAHAAQEA(A)R in which the E and Q positions with respect to the radical site were exchanged (Table 1). The data show that the side-chain eliminations are most abundant in z_5 – z_7 ions (52 %–69 % of the total fragment ion intensities) where the C_α radical site is next to or one residue away from E or Q, and then decrease as the radical site is moved farther away along the peptide backbone. Proximity effects were observed for the competitive elimination from the E and Q residues in the EQ and QE sequence doublets. The z_5 ions, in particular, showed a substantial preference for combined eliminations from E over those from Q (9:1) in $^{\bullet}\text{AEQAR}$, and conversely from Q over E (2.8:1) in

$^{\bullet}\text{AQEAR}$. This preference was lower in dissociations of the C-terminal homologous z_6 ions where eliminations from E were more abundant than those from Q (2.3:1) in $^{\bullet}\text{AEQAAR}$, and conversely from Q over E (2.4:1) in $^{\bullet}\text{AQEAAR}$. Note that both the z_5 ions from the nonapeptides and z_6 ions from the decapeptides must undergo the same type of H atom transfers to trigger the side chain loss. The different distributions of the losses from the E and Q chains may be due to different internal solvation in $^{\bullet}\text{AEQAR}$ and $^{\bullet}\text{AEQAAR}$ that affects the transition state energies and thus the kinetics of the competing H-atom transfers. The sequence preference further indicates that the intermediate C_α radicals at the E and Q residues have similar energies and their formation is controlled kinetically rather than thermodynamically. A similar argument can be made for the C_γ radicals at E and Q, which also show positional dependence that is a kinetic rather than thermodynamic effect.

An interesting, albeit less straightforward, effect was observed for the competing eliminations of CH_2COOH and $\text{CH}_2 = \text{CHCOOH}$ from E and CH_2CONH_2 and $\text{CH}_2 = \text{CHCONH}_2$ from Q. The z_5 $^{\bullet}\text{AEQAR}$ ion showed a

Table 1. Relative Intensities of Side-Chain Loss Fragments from z Ions

Relative Intensity (% of sum of fragment ions)																
		AAHAAEQAX"								AAHAAQEAAX"						
		-CH ₂ COX		-CH ₂ =CHCOX		Side-chain loss				-CH ₂ COX		-CH ₂ =CHCOX		Side-chain loss		
n	Ion X"	X = OH	NH ₂	OH	NH ₂	OH	NH ₂	all		OH	NH ₂	OH	NH ₂	OH	NH ₂	all
1	z ₅ R	39.2	4.6	11.9	0.9	51.1	5.5	56		11.7	40.2	6.5	10.6	18.2	50.8	69
	z ₆ AR	33.9	10.3	7.9	4.0	41.8	14.2	56		12.2	28.2	4.7	6.8	16.9	34.7	52
2	z ₆ R	19.8	15.9	4.0	12.7	23.9	28.6	52		15.4	14.7	21.8	0.8	37.2	15.5	53
	z ₇ AR	23.0	20.4	3.7	9.2	26.7	29.6	56		15.6	22.2	14.2	1.8	29.8	24.0	54
3	z ₇ R	12.5	16.8	3.5	4.2	16.0	21.0	37		20.3	10.3	5.1	2.2	25.4	12.5	38
	z ₈ AR	12.5	13.6	4.0	12.5	16.6	26.1	43		14.7	7.6	7.5	1.7	22.2	9.3	32
4	z ₈ R	8.1	9.3	7.2	3.6	15.3	12.9	28		10.7	2.0	9.0	7.5	19.7	9.5	29
	z ₉ AR	6.7	9.4	8.8	4.8	15.4	14.2	30		8.0	6.0	10.2	11.9	18.1	17.9	36
5	z ₉ R	9.5	8.1	10.5	5.8	20.0	13.9	34		6.7	9.7	4.8	12.2	11.4	21.9	33
	z ₁₀ AR	8.8	10.2	9.3	6.2	18.1	16.4	35		8.3	13.1	6.4	12.0	14.7	25.1	40

3.3:1 preference for loss of CH_2COOH over loss of $\text{CH}_2=\text{CHCOOH}$, and the z_5 $^{\bullet}\text{AEQAR}$ ion showed a 3.8:1 preference for loss of CH_2CONH_2 over loss of $\text{CH}_2=\text{CHCONH}_2$. These eliminations require different cyclic transition states for the hydrogen transfer, which is a 5-membered TS for the H_α -atom transfer triggering loss of CH_2COX , and a 7-membered TS for the H_γ transfer triggering loss of $\text{CH}_2=\text{CHCOX}$. Five-membered TS for H-atom transfers between neighboring C_α positions in z ions have been studied and found to require low energy barriers (e.g., 58 kJ mol^{-1}) [12]. The fact that the H_γ -atom transfers through 7-membered TS are competitive in $^{\bullet}\text{AEQAR}$ and $^{\bullet}\text{AEQAR}$ indicates that these rearrangements must require comparatively low energies. An important aspect here is that the energy barriers for the H_α and H_γ atom transfers are in the same range as those for amide trans $\rightarrow$ cis rotations in C_α radicals [12], and not much higher than the barriers for other conformational transformations of the backbone and side chains. This points to the importance of conformational changes accompanying H-atom transfers in radicals of the z -ion type.

Conformational changes including amide trans $\rightarrow$ cis rotations presumably played a role in dissociations of z ions in which the C_α radical center was farther away from the E and Q residues (Table 1). Thus, CH_2COOH elimination was slightly favored over CH_2CONH_2 elimination from z_6 $^{\bullet}\text{AAEQAR}$ and z_7 $^{\bullet}\text{AAEQAAR}$ ions, whereas $\text{CH}_2=\text{CHCONH}_2$ elimination was favored over $\text{CH}_2=\text{CHCOOH}$ elimination from the same ions. Conversely, $\text{CH}_2=\text{CHCOOH}$ elimination was favored over $\text{CH}_2=\text{CHCONH}_2$ elimination from z_6 $^{\bullet}\text{AAQEAR}$ and z_7 $^{\bullet}\text{AAQEAAR}$ ions. These eliminations require larger cyclic transition states for the activating H_α and H_γ transfers. Thus, 8- and 11-membered transition states are needed for the transfer of the H_α and H_γ , respectively, from the closer E or Q residue, and 11- and 14-membered transition states are involved in the transfer of the H_α and H_γ , respectively, from the more remote E or Q residue. For side-chain dissociations of the larger z ions see Table 1. It should be noted that the hydrogen transfers preceding the side-chain eliminations presumably may involve consecutive H_α atom migrations between neighboring amino acid residues, which have low activation energies (vide supra) and would bring the C_α radical center closer to the E or Q side chain. Such a mechanism does not appear fully compatible with the different side-chain loss selectivity in $^{\bullet}\text{AEQAR}$ and, for example, z_8 $^{\bullet}\text{AHAAEQAR}$ (Table 1). However, if a series of H_α atom migrations occurred in $^{\bullet}\text{AHAAEQAR}$, moving the radical center to form $\text{AHA}^{\bullet}\text{AEQAR}$, the latter would be electronically and conformationally different from $^{\bullet}\text{AEQAR}$ and, hence, could have a different reactivity in initiating the side-chain dissociations. Interpretation of the competing dissociations would require mapping the potential energy surface, locating the transition states, and calculating the rate constants for the

competing reactions. We will provide relevant experimental and computational data to treat the kinetics of competitive dissociations of several selected pentapeptide z ions in Part 2 of this work.

Conclusions

The results of this study of z -ions from systematically developed sets of penta-, nona-, and decapeptides allow us to arrive at the following conclusions. z -Ions containing amino acid residues with readily transferrable side-chain β -hydrogen atoms (F, Y, H, V, W) undergo very facile radical-induced backbone cleavages. These compete with side-chain dissociations in D, E, N, Q, L, K, S, R, C, and M that are triggered by transfers of α - and γ -hydrogen atoms. Competing H_α and H_γ transfers in z ions containing the E and Q residues show dependence on the residue position relative to the initial C_α radical site.

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References

1. Tureček, F. N- C_α bond dissociation energies and kinetics in amide and peptide radicals. Is the dissociation a nonergodic process? *J. Am. Chem. Soc.* 2003, **125**, 5954–5963
2. Chung, T. W.; Tureček, F. Proper and improper aminoketyl radicals in electron-based peptide dissociations. *Int. J. Mass Spectrom.* 2011, **301**, 55–61
3. Zubarev, R. A.; Kelleher, N. L.; McLafferty, F. W. Electron capture dissociation of multiply charged protein cations. A nonergodic process. *J. Am. Chem. Soc.* 1998, **120**, 3265–3266.
4. Syka, J. E. P.; Coon, J. J.; Schroeder, M. J.; Shabanowitz, J.; Hunt, D. F. Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. *Proc. Natl. Acad. Sci. U.S.A.* 2004, **101**, 9528–9533
5. Li, W.; Song, C.; Bailey, D. J.; Tseng, G. C.; Coon, J. J.; Wysocki, V. H. Statistical Analysis of Electron Transfer Dissociation Pairwise Fragmentation Patterns. *Anal. Chem.* 2011, **83**, 9540–9545
6. Leymarie, N.; Costello, C. E.; O'Connor, P. B. Electron capture dissociation initiates a free radical reaction cascade. *J. Am. Chem. Soc.* 2003, **125**, 8949–8958
7. Afonso, C.; Tabet, J.-C.; Giorgi, G.; Tureček, F. Gas-phase doubly charged complexes of cyclic peptides with copper in +1, +2, and +3 formal oxidation states. Formation, structures, and electron capture dissociation. *J. Mass Spectrom.* 2012, **47**, 208–220
8. Kruger, N. A.; Zubarev, R. A.; Horn, D. M.; McLafferty, F. W. Electron capture dissociation of multiply charged peptide cations. *Int. J. Mass Spectrom.* 1999, **185/186/187**, 787–793
9. Liu, J.; Liang, X.; McLuckey, S. A. On the value of knowing a z' ion for what it is. *J. Proteome Res.* 2008, **7**, 130–137

10. Xia, Y.; Han, H.; McLuckey, S. A. Activation of intact electron-transfer products of polypeptides and proteins in cation transmission mode ion/ion reactions. *Anal. Chem.* 2008, **80**, 1111–1117
11. Swaney, D. L.; McAlister, G. C.; Wirtala, M.; Schwartz, J. C.; Syka, J. E. P.; Coon, J. J. Supplemental activation method for highly efficient electron transfer dissociation of doubly protonated peptide precursors. *Anal. Chem.* 2007, **79**, 477–485
12. Chung, T. W.; Tureček, F. Backbone and side chain-specific dissociations of *z* ions from nontryptic peptides. *J. Am. Soc. Mass Spectrom.* 2010, **21**, 1279–1295
13. Tureček, F.; Chung, T. W.; Moss, C. L.; Wyer, J. A.; Ehlerding, A.; Holm, A. I. S.; Zettergren, H.; Nielsen, S. B.; Hvelplund, P.; Chamot-Rooke, J.; Bythell, B.; Paizs, B. The histidine effect. Electron transfer and capture cause different dissociations and rearrangements of histidine peptide cation radicals. *J. Am. Chem. Soc.* 2010, **132**, 10728–10740
14. Van der Rest, G.; Hui, R.; Frison, G.; Chamot-Rooke, J. Dissociation channel dependence on peptide size observed in electron capture dissociation of tryptic peptides. *J. Am. Soc. Mass Spectrom.* 2011, **22**, 1631–1644
15. Moss, C. L.; Liang, W.; Li, X.; Tureček, F. The early life of a peptide cation radical. Ground and excited-state trajectories of electron-based peptide dissociations during the first 330 femtoseconds. *J. Am. Soc. Mass Spectrom.* 2012, **23**, 446–459
16. Holm, A. I. S.; Hvelplund, P.; Kadhane, U.; Larsen, M. K.; Liu, B.; Nielsen, S. B.; Panja, S.; Pedersen, J. M.; Skydstrup, T.; Stochkel, K.; Williams, E. R.; Worm, E. S. On the mechanism of electron-capture-induced dissociation of peptide dications from ¹⁵N labeling and crown-ether complexation. *J. Phys. Chem. A* 2007, **111**, 9641–9643
17. Cooper, H. J.; Hudgins, R. R.; Haksansson, K.; Marshall, A. G. Characterization of amino acid side chain losses in electron capture dissociation. *J. Am. Soc. Mass Spectrom.* 2002, **13**, 241–249
18. Sun, Q.; Nelson, H.; Stolz, B. M.; Julian, R. R. Side chain chemistry mediates backbone fragmentation in hydrogen deficient peptide radicals. *J. Proteome Res.* 2009, **8**, 958–966
19. McClellan, J. E.; Murphy, J. P. III; Mulholland, J. J.; Yost, R. A. Effects of fragile ions on mass resolution and on isolation for tandem mass spectrometry in the quadrupole ion trap mass spectrometer. *Anal. Chem.* 2002, **74**, 402–412
20. Swaney, D. L.; McAlister, G. C.; Wirtala, M.; Schwartz, J. C.; Syka, J. E. P.; Coon, J. J. Supplemental activation method for high-efficiency electron transfer dissociation of doubly protonated peptide precursors. *Anal. Chem.* 2007, **79**, 477–485
21. Wysocki, V. H.; Tsapralis, G.; Smith, L. L.; Brei, L. A. Mobile and localized protons: a framework for understanding peptide dissociation. *J. Mass Spectrom.* 2000, **35**, 1399–1406
22. Tureček, F.; Syrstad, E. A. Mechanism and energetics of intramolecular hydrogen transfer atom transfer in amide and peptide radicals and cation-radicals. *J. Am. Chem. Soc.* 2003, **125**, 3353–3369
23. Hao, C.; Seymour, J. L.; Tureček, F. Electron super-rich radicals in the gas phase. A neutralization-reionization mass spectrometric and ab initio/RRKM study of diaminohydroxymethyl and triaminomethyl radicals. *J. Phys. Chem. A* 2007, **111**, 8829–8843
24. Hayakawa, S.; Matsubara, H.; Panja, S.; Hvelplund, P.; Nielsen, S. B.; Chen, X.; Tureček, F. Experimental evidence for an inverse hydrogen migration in arginine radicals. *J. Am. Chem. Soc.* 2008, **130**, 7645–7654
25. Panja, S.; Nielsen, S. B.; Hvelplund, P.; Tureček, F. Inverse hydrogen atom migration in arginine-containing peptide ions upon electron transfer. *J. Am. Soc. Mass Spectrom.* 2008, **19**, 1726–1742
26. Savitski, M. M.; Kjeldsen, F.; Nielsen, M. L.; Zubarev, R. A. Hydrogen rearrangement to and from radical *z* fragments in electron capture dissociation of peptides. *J. Am. Soc. Mass Spectrom.* 2007, **18**, 113–120.
27. Ledvina, A. R.; McAlister, G. C.; Gardner, M. W.; Smith, S. I.; Madsen, J. A.; Schwartz, J. C.; Stafford, G. C. Jr.; Syka, J. E. P.; Brodbelt, J. S.; Coon, J. J. Infrared photoactivation reduces peptide folding and hydrogen-atom migration following ETD tandem mass spectrometry. *Angew. Chem. Int. Ed.* 2009, **48**, 8526–8528
28. Byers, J. H.; Campbell, J. E.; Knapp, F. H.; Thissell, J. G. Radical aromatic substitution via atom-transfer addition. *Tetrahedron Lett.* 1999, **40**, 2677–2680
29. Walden, S. E.; Wheeler, R. A. Distinguishing features of indolyl radical and radical cation: implications for tryptophan radical studies in proteins. *J. Phys. Chem.* 1996, **100**, 1530–1535
30. Gregersen, J. A.; Tureček, F. Mass-spectrometric and computational study of tryptophan radicals (Trp + H)[•] produced by collisional electron transfer to protonated tryptophan in the gas phase. *Phys. Chem., Chem. Phys.* 2010, **12**, 13434–13447
31. Moran, D.; Jacob, R.; Wood, G. P. F.; Coote, M. L.; Davies, M. J.; O'Hair, R. A. J.; Easton, C. J.; Radom, L. Rearrangements in model peptide-type radicals via intramolecular hydrogen-atom transfer. *Helv. Chem. Acta* 2006, **89**, 2254–2271